Table 1. Primers used in this study.

Primer name	Sequence (5′ → 3′)
ExfruF	ATCAGTAGTACTGTACGTCGTGTGC
ExfruR	GATCTTGCCGATCCCCGTCGATCGC
ExfrdF	GTGTTGTTCAAGTTGGGTGCATGTG
ExfrdR	TCTTGAAGGCGAGTAGACGGACTCA
ExfuF	GTTCAACTTTGGTTTTGTGGAGGAG
ExfuR	CCTTCTCCCTCTTCCTACCGTCCTC
hphF	CTCCGGAGTTGAGACAAATGG
hphR	CATCCACTGCACCTCAGAGC

P008

Molecular mechanisms associated with fluconazole resistance and genetic diversity in clinical *Candida krusei* isolates from North India

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Objectives: *Candida krusei* accounts for 2.8% of invasive candidiasis worldwide. Fluconazole resistance and its underlying mechanism in clinical isolates of *C. krusei* ($n = 137$) collected from eight hospitals in India were investigated. Also, genetic diversity of *C. krusei* strains among different hospitals was studied through short tandem repeat (STR) genotyping.

Material and Method: All the isolates were identified by MALDI-TOF MS. Antifungal susceptibility test was done by using broth microdilution method (CLSI-M27). To evaluate the genetic relatedness among the strains, STR typing was done by using 9 STR markers. To understand the fluconazole-resistant mechanisms in *C. krusei*, known fluconazole resistance mechanisms such as alterations in target enzyme ERG11 and drug transporters ABC1, and ABC2 were investigated in 35 *C. krusei* isolates [18 fluconazole-susceptible (FLU-S), and 17 fluconazole-susceptible dose-dependent (FLU-SDD)]. Furthermore, transcriptomics of one FLU-SDD (MIC 32 mg/L) and one FLU-S (MIC 4 mg/l) isolate was performed.

Results: Majority (77%) of *C. krusei* isolates were from bloodstream infections. Notably, 70% of candidemia cases occurred in neonatal intensive care units (NICUs). Remarkably, 81% ($n = 110$) were detected as fluconazole-SDD (MIC 16-32 mg/l), and the remaining 19% were FLU-S (MIC ≤ 8 mg/l). Marked genetic diversity with 51 diverse STR types was noticed among the 106 isolates. Interestingly, two ongoing candidemia outbreaks were observed in two geographically separated hospitals both representing NICU isolates. In addition, a large cluster containing isolates from six different hospitals was observed. ERG11 mutation analysis revealed that it did not harbor any mutation contributing to the flu-resistance. Overexpression of the ABC1 gene in 11 FLU-SDD isolates out of 17 as compared to FLU-S isolates was noted. However, no alteration was observed in the expression of ERG11 and ABC2 in both groups.

Transcriptomics analysis revealed a significant number of differentially regulated genes were distributed in various gene-ontology terms including transport (10 genes), mitogen-activated protein kinase (MAPK) signaling (8 genes, MSG5, PTP3, STE50, BNR1, OPY2, STE5, SKN7, and RLM1), ergosterol biosynthesis (3 genes, ERG24, ERG25, and ERG26) and transcription factors (7 genes). In addition to the up-regulation of ergosterol pathway genes, overexpression of key transcriptional regulator of ergosterol biosynthesis genes UPC2 was observed in FLU-SDD isolates as compared with susceptible. Additionally, FLU-SDD isolate showed 2-fold increased expression of PDR12, plasma membrane ATP-binding cassette (ABC) transporter. Next, ICL1 (Isocitrate Lyase), a major glyoxylate-synthesizing enzyme was found to be 5-fold down-regulated in FLU SDD isolate compared to susceptible. The loss of ICL1 alters the expression of the FKS1, ERG11, and CDR2 genes in *C. albicans*. Taken together, the increased expression of PDR12 and altered MAPK singling network may partially account for the FLU resistance in *C. krusei* FLU-SDD isolate.

Conclusion: *Candida krusei* isolates among different hospitals showed large genetic diversity (54 different genotypes). Also, the presence of *C. krusei* clonal strains in six different hospitals suggests possible introduction from a widespread environmental source and human-to-human transmission. In comparison to other *Candida* species, the resistant mechanism in *C. krusei* seems to be more complex. Therefore, an in-depth study of other resistance mechanism pathways in *C. krusei* is further warranted.

P009

Investigation of *in vitro* antifungal susceptibility testing and genetic diversity of clinical isolates of *Trichophyton benhamiae* and *Trichophyton eriotrephon* in Iran

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Background: *Trichophyton benhamiae* is a zoophilic dermatophyte, known as one of the causative agents of dermatophytosis.

Objectives: The purpose of this study was to explore the genotypes of *T. benhamiae* strains isolated from geographically different areas of Iran and also to evaluate *in vitro* antifungal susceptibility profile of these strains against seven antifungal drugs.

Methods: A total of 22 strains of *T. benhamiae* and 2 strains of *T. eriotrephon* were isolated from patients with distinct types of dermatophytosis. DNA extraction and amplification of rDNA regions using ITS1 and ITS4 primers were conducted on the isolates. The *in vitro* antifungal susceptibility of posaconazole (PSC), voriconazole (VRC), itraconazole (ITC), ketoconazole (KET), caspofungin (CAS), terbinafine (TRB) and griseofulvin (GRZ) was evaluated according to CLSI M38-A2 protocol.

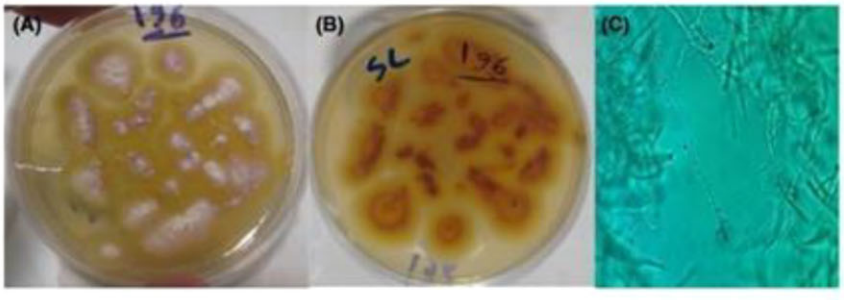
Results: The multiple alignments of the ITS-rDNA sequences of *T. benhamiae* indicated a mean similarity of 99.5%, with 0-3 interspecies nucleotide differences. The geometric mean (GM) values of minimum inhibitory concentrations (MICs) and minimum effective concentrations (MECs) across the all isolates were respectively: TRB: 0.025 mg/l, PSC: 0.032 mg/l, ITC: 0.050 mg/l, and VRC: 0.059 mg/l with lower values and CAS: 0.31 mg/l, KTZ: 0.56 mg/l, and GRZ: 0.76 mg/l with higher values.

Conclusion: Diverse ITS sequence types of *T. benhamiae* were shown in different geographical regions of Iran. The TRB, PSC, and ITC were the most effective drugs against *T. benhamiae* strains, respectively. Furthermore, in our study, two strains of *T. eriotrephon* as a scarce dermatophyte species were described.

Geometric mean of MICs/MECs, MIC/MEC ranges and MIC/MEC50 and MIC/MEC90 values obtained by testing the susceptibility of 22 Iranian *T. benhamiae* isolates to antifungal agents

	PSC	ITC	VRC	KET	TRB	GRZ	CAS
GM (mg/L)	0.032	0.050	0.059	0.56	0.025	0.76	0.31
Min(mg/L)	0.008	0.016	0.016	0.063	0.008	0.125	0.125
Max(mg/L)	0.125	0.125	0.25	4	0.125	4	0.5
MIC/MEC50(mg/L)	0.032	0.063	0.063	0.5	0.032	1	0.25
MIC/MEC90(mg/L)	0.063	0.063	0.125	2	0.063	2	0.5
T. eriotrephon (KP789415)	0.063	0.063	0.125	0.5	0.063	0.5	0.25
T. eriotrephon (KP789416)	0.063	0.032	0.125	0.25	0.063	0.5	0.125

Abbreviations: CAS, caspofungin; GRZ, griseofulvin; ITC, itraconazole; KET, ketoconazole; MEC, minimum effective concentration; MIC, minimum inhibitory concentration; MIC50, minimal concentration that inhibits 50% of isolates; MIC90, minimal concentration that inhibits 90% of isolates; PSC, posaconazole; TRB, terbinafine; VRC, voriconazole.



The surface (A) and reverse (B) appearance on Mycobiotic Agar following 10-day incubation at 27°C, and the microscopic morphology (C) of *T. eriotrephon* isolated from patient with tinea manuum